Received: 12 May 2023 | Revised: 23 June 2023 | Accepted: 10 July 2023

DOI: 10.1002/psp4.13011

REVIEW

Model-informed drug development of autologous CAR-T cell therapy: Strategies to optimize CAR-T cell exposure leveraging cell kinetic/dynamic modeling

Anna M. Mc Laughlin[1] | Peter A. Milligan[1] | Cassian Yee[2,3] | Martin Bergstrand[1]

[1]Pharmetheus AB, Uppsala, Sweden

[2]Department of Melanoma Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA

[3]Department of Immunology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA

Correspondence
Anna M. Mc Laughlin, Pharmetheus AB, Dag Hammarskjölds väg 36b, 75237 Uppsala, Sweden.
Email: anna.mclaughlin@pharmetheus.com

Abstract

Autologous Chimeric antigen receptor (CAR-T) cell therapy has been highly successful in the treatment of aggressive hematological malignancies and is also being evaluated for the treatment of solid tumors as well as other therapeutic areas. A challenge, however, is that up to 60% of patients do not sustain a long-term response. Low CAR-T cell exposure has been suggested as an underlying factor for a poor prognosis. CAR-T cell therapy is a novel therapeutic modality with unique kinetic and dynamic properties. Importantly, "clear" dose-exposure relationships do not seem to exist for any of the currently approved CAR-T cell products. In other words, dose increases have not led to a commensurate increase in the measurable in vivo frequency of transferred CAR-T cells. Therefore, alternative approaches beyond dose titration are needed to optimize CAR-T cell exposure. In this paper, we provide examples of actionable variables – design elements in CAR-T cell discovery, development, and clinical practice, which can be modified to optimize autologous CAR-T cell exposure. Most of these actionable variables can be assessed throughout the various stages of discovery and development as part of a well-informed research and development program. Model-informed drug development approaches can enable such study and program design choices from discovery through to clinical practice and can be an important contributor to cell therapy effectiveness and efficiency.

INTRODUCTION

Adoptive cell therapy describes the administration of immune cells that recognize and eliminate target cells, such as tumor cells. Chimeric antigen receptor (CAR)-T cells are adoptively transferred engineered immune cells, which have been genetically modified to express an artificial receptor recognizing a surface antigen on the target cell. Six autologous CAR-T cell products, all targeting hematological tumors via the CD19 or B-cell maturation antigen (BCMA), have so far been approved by the US Food and Drug Administration (FDA; Table 1).

The CAR-T cell treatment process largely consists of five steps: In the first step, T cells are extracted from a patient's blood via apheresis. In the second step, the T cells are genetically modified to express a CAR which

TABLE 1 Overview of FDA-approved autologous CAR-T cell products.

CAR-T cell product	Manufacturer	Target and co-stimulatory domain	FDA-approved indication	Year of FDA approval	Comments
Tisagenlecleucel (Kymriah)	Novartis	CD19 41BB	r/r ALL (≤25 years) r/r LBCL (adults) r/r FL (adults)	2017	First approved CAR-T cell therapy
Axicabtagene ciloleucel (Yescarta)	Kite/Gilead Sciences	CD19 CD28	r/r LBCL (adults) r/r FL (adults)	2017	First approved CAR-T cell therapy for adults
Brexucabtagene autoleucel (Tecartus)	Kite/Gilead Sciences	CD19 CD28	r/r MCL (adults) r/r ALL (adults)	2020	Enrichment of T cells during manufacturing to avoid contamination with tumor cells which could lead to activation, expansion, and exhaustion during ex vivo expansion
Lisocabtagene maraleucel (Breyanzi)	Juno/BMS	CD19 41BB	r/r LBCL (adults)	2021	$CD4^+$ and $CD8^+$ CAR-T cells are manufactured separately and later combined in a 1:1 ratio
Idecabtagene vicleucel (Abecma)	Celgene/BMS	BCMA 41BB	r/r MM (adults)	2021	Binds to one epitope of BCMA
Ciltacabtagene autoleucel (Carvykti)	Janssen	BCMA 41BB	r/r MM (adults)	2022	Binds to two epitopes of BCMA, resulting in increased avidity

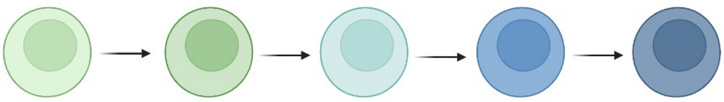
Abbreviations: ALL, acute lymphoblastic leukemia; BCMA, B-cell maturation antigen; BMS, Bristol-Myers Squibb; FDA, US Food and Drug Administration; FL, follicular lymphoma; LBCL, large B-cell lymphoma; MM, multiple myeloma; r/r, relapsed/refractory.

recognizes the target antigen. In the third step, the newly generated cells are activated and expanded ex vivo to generate a high number of CAR-T cells. In the fourth step, patients receive a pre-conditioning lymphodepleting chemotherapy regimen. This creates beneficial survival conditions for the CAR-T cells, which are infused in the fifth step.

CAR-T cells have unique kinetic properties,[1,2] which are different from any other currently approved therapeutic modality. Their kinetic profile can be separated into three distinct phases: after infusion, CAR-T cells distribute out of the peripheral blood and into tissue, observable as a transient decline in peripheral blood concentrations (distribution phase). Upon binding to their target, CAR-T cells release cytotoxic substances, which kill tumor cells. At the same time, they rapidly and extensively proliferate until they reach their peak expansion after ~7–14 days (proliferation phase). This expansion phase is followed by a period of biphasic decline, with the two phases resulting from the presence of different CAR-T cell phenotypes with different life spans (contraction phase).

The CAR-T cell phenotypes are defined based on their expression of cell surface markers (mainly CCR7, CD28, CD95, and CD45RO), which can be identified via flow cytometry. Different phenotypes show different expansion, survival, and cytotoxicity characteristics (Figure 1). According to the widely accepted progressive differentiation T cell model,[3] describing the relationship between effector and memory T cells, T cells differentiate upon stimulation (e.g., by contact with their antigen). The least differentiated naïve T cells (T_N) are postulated to differentiate into stem cell memory T cells (T_{SCM}), which again differentiate into central memory T cells (T_{CM}). The T_{CM} then differentiate into effector memory T cells, which finally differentiate into effector T cells. With increasing differentiation, T cells lose their expansion potential and fitness, while increasing their cytotoxicity.[3] T cells are further distinguished into $CD8^+$ subsets with cytotoxic properties, and $CD4^+$ subsets with helper functions.[4]

CAR-T cell therapy has revolutionized the treatment of relapsed/refractory (R) hematological malignancies. Initial response rates of up to 100%[5] have been achieved,



	Naive T cells (T_N)	T memory stem cells (T_{SCM})	Central memory T cells (T_{CM})	Effector memory T cells (T_{EM})	Effector T cells (T_{Eff})
Surface markers for subtype identification	CCR7+ CD28+ CD95- CD45RO-	CCR7+ CD28+ CD95+ CD45RO-	CCR7+ CD28+ CD95+ CD45RO+	CCR7- CD28- CD95+ CD45RO+	CCR7- CD28- CD95+ CD45RO-
Proliferation potential	High	High	High	Moderate	Low
Cytotoxicity	Low	Low	Moderate	Moderate - high	High

FIGURE 1 CAR-T cell phenotypes and their relevant characteristics in the context of adoptive cell therapy. Created with BioRender.com.

yet a durable response is only observed in as few as 40%[6] of patients. Several factors, such as a high initial tumor burden,[7] an immunosuppressive tumor microenvironment,[8] low CAR-T cell maximum concentration (C_{max}),[9,10] and persistence (measured as small area under the concentration-time curve [AUC])[11] have been associated with poor outcomes. Tumor eradication may also be affected by stochastic processes at low tumor cell counts, which could be a potential cause for treatment evasion.[12]

Some of these factors, such as the initial tumor size and a patient's tumor microenvironment, are difficult to modify. In contrast, the negative effect of a low CAR-T cell exposure should, at least in theory, be modifiable using dose titration. Unfortunately, "clear" dose-exposure relationships do not seem to exist for many of the currently approved CAR-T cell products. In a recent review on the pharmacology of CAR-T cells, no clear relationship between dose and exposure was identified for three currently approved CAR-T cell products targeting the CD19 antigen (tisagenlecleucel, axicabtagene ciloleucel, and lisocabtagene maraleucel), whereas only positive trends were identified for two products (brexucabtagene autoleucel [anti-CD19] and idecabtagene vicleucel [anti-BCMA]).[2] Supporting these findings, a dedicated randomized dose optimization study of CART-19 for the treatment of r/r $CD19^+$ chronic lymphocytic leukemia observed neither higher exposure nor significantly longer survival in patients receiving a high CAR-T cell dose (5×10^8 cells) compared to patients receiving a low CAR-T cell dose (5×10^7 cells).[9] Similarly, a recent dedicated model-based exposure-response analysis for idecabtagene vicleucel did not find a dose-exposure relationship.[10] This paper discusses actionable variables in the autologous CAR-T cell drug discovery and development, which may be modified to optimize CAR-T cell exposure in the absence of dose-exposure relationships (Table 2).

Model-informed drug development (MIDD)[13,14] has been defined as an approach that develops and applies exposure-based, biological, and statistical models derived from across preclinical and clinical data sources to inform drug development strategies and decision making. It aims to integrate information from diverse data sources and to generate information that would be difficult to obtain experimentally.[15] The ability to decrease the level of compound/study/program uncertainty can both lower the cost of program failures and improve the overall indication success rates. As the cost and morbidity of CAR-T cell therapy can be significant, product optimization strategies in the early drug discovery and drug development phases become especially relevant in the absence of clear dose-exposure and dose–response relationships. Model-informed approaches have the potential to improve the efficiency of all steps in the knowledge generating cycle (Figure 2): For example, optimized experimental and study designs can be proposed based on clinical trial simulations and/or optimal design theory,[16,17] model-based interim analysis can be used as a basis for adaptive study design, and model-based algorithms can be used to individualize the studied therapy. In the analysis of data from longitudinal studies, the model-based approaches offer a multitude of benefits that all contribute to generating more information from the available data. Perhaps most importantly, the MIDD paradigm allows one to integrate different sources of information and translate it into actionable knowledge including a quantitative measure of the associated uncertainty. This ultimately leads to better informed decisions throughout the entire drug development process.

Several studies have pointed toward the phenotype (Figure 1) and functional state of CAR-T cells as being of highest relevance for their in vivo expansion and antitumor activity. In a recent study exploring the relationship between the phenotypic composition of commercial anti-CD19 CAR-T cell products and exposure as well as outcome, the in vivo CAR-T cell expansion, disease response, and progression-free survival of patients with large B-cell lymphoma were significantly associated with

TABLE 2 Overview of proposed actionable variables in MIDD of CAR-T cells.

Development stage	Actionable variable	Issues addressed	Key indicators of success
Drug discovery CAR design	Optimization of the CAR affinity	Affinity-efficacy relationships seem to follow a bell-shaped form; affinity should be optimized to assure sufficient activation with minimal activation-induced cell-death and/or on-target off-tumor toxicity	High in vitro and preclinical CAR-T cell expansion as well as sustained cytokine production and cytotoxicity, minimal on-target off-tumor activity
Drug discovery CAR design	Selection and optimization of the CAR hinge/spacer domain	A minimum distance between the CAR and the target cell seems to be required for optimal efficacy. The expression of target epitopes with respect to the target cell membranes is variable, resulting in variable needs for spacers	High in vitro and preclinical CAR-T cell expansion, cytokine production, and cytotoxicity
Drug discovery CAR design	Selection of the CAR co-stimulatory domain(s)	A co-stimulatory domain as part of the CAR design is needed for sufficient and sustained expansion. Several domains exist and the integration of more than one domain is possible	High in vitro and preclinical CAR-T cell expansion with low proneness to exhaustion induced by tonic signaling
Drug discovery CAR design	Selection and optimization of CAR element combinations	CAR-T cell elements may show varying properties depending on their combination with other cell elements	Optimal in vitro and preclinical CAR-T cell expansion, cytokine production, cytotoxicity, and fitness
Drug development Manufacturing	Stimulation of cells during the ex vivo expansion (choice and dose of antigen-presenting antibodies/cells and in vitro activation)	Different types of antigen-presenting antibodies or cells seem to have different effects on CAR-T cell expansion and phenotype shift in a dose-dependent manner	High frequency of less-differentiated CAR-T cell phenotypes with high expansion potential after ex vivo expansion
Drug development Manufacturing	Optimization of the duration of the ex vivo expansion	The number of generated CAR-T cells increases with increasing expansion duration. At the same time, longer expansion durations lead to increased CAR-T cell differentiation, resulting in an overall less potent product	Sufficient number of CAR-T cells with good fitness and high expansion potential after ex vivo expansion
Drug development Manufacturing	Addition of cytokines to the ex vivo expansion medium and their concentrations	Different cytokines are used to stimulate ex vivo expansion, but their influence on CAR-T cell phenotype differentiation varies. Moreover, the impact of cytokines seems to be dose–/concentration-dependent	High frequency of less-differentiated CAR-T cell phenotypes with high expansion potential after ex vivo expansion
Clinical practice Bridging	Selection of patients who might benefit from bridging therapy and optimization of such treatment	Some patients require bridging therapy to control their disease during the CAR-T cell manufacturing period, however mixed results on the effect of bridging therapy have been reported	Successful disease control, high in vivo expansion, improved survival
Clinical practice Pre- and/or post-infusion immune-modulation	Optimization of the preconditioning lymphodepleting chemotherapy; potential application of post-infusion cytokines	Exposure-response relationships have been identified for both drugs included in the most common lymphodepleting chemotherapy regimen. There seems to be the possibility for further regimen optimization based on a patient's tumor burden and potentially CAR-T cell dose	High in vivo expansion, improved survival
Clinical practice Combination therapy	Combination of CAR-T cells with other treatments	Ongoing investigations indicate the potential for combination treatments to result in increased efficacy	High in vivo expansion, improved survival

Abbreviation: MIDD, model-informed drug development.

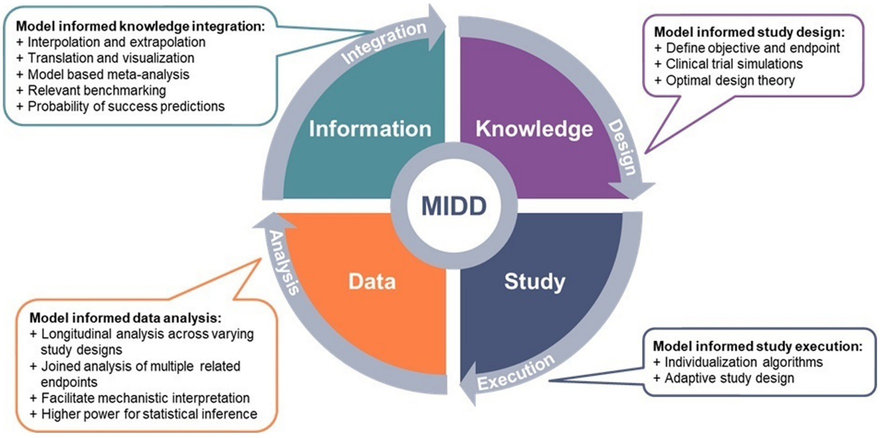


FIGURE 2 Schematic overview of model-informed drug development (MIDD) elements.

the presence of CAR-T cells with a $CD8^+$ central memory phenotype.[18] This most recent finding is supported by other studies identifying a high frequency of CAR-T cells with a naïve- or memory-like phenotype as positive predictors for a clinical response.[19–21] Unfortunately, T cells in patient apheresis products (used as starting material for CAR-T cell manufacturing) often show low fitness and high frequencies of a highly differentiated effector phenotype.[22] Thus, one of the most relevant goals of the CAR-T cell product optimization process across drug discovery and drug development is the increase and/or preservation of a high frequency of CAR-T cells with an early differentiation phenotype (e.g., naïve, memory stem, or central memory).

DRUG DISCOVERY

A CAR is an artificial receptor construct consisting of several components. First-generation CARs, consisting of an extracellular antigen-binding domain and an intracellular CD3ζ-signaling domain, showed low expansion and a lack of long-term persistence.[23,24] Second-generation CARs, which are used in all currently approved CAR-T cell products, therefore incorporate an additional intracellular co-stimulatory domain resulting in improved expansion and persistence properties. The extracellular domain, usually a single-chain fragment variable (scFv) originating from a monoclonal antibody, is linked to intracellular domains via hinge/spacer and transmembrane domains (Figure 3). The selection of each of the receptor's components can have a significant impact on the functional properties of the final cell product.

CAR design: Antigen-binding domain

The activation threshold of a CAR depends on the antigen-binding domain's affinity and avidity, as well as on the density of the expressed target antigen.[25] High affinity scFvs are often selected to assure sufficient activation; however, they might not always be the optimal choice. First, if the target antigen is not exclusive to the tumor but also expressed at low levels on healthy tissue, the use of a high affinity scFv may result in life-threatening on-target off-tumor toxicity. Second, CAR-T cells with high affinity scFvs have been reported to show lower efficacy and proliferation compared to cells with low to medium affinity,[26] most likely due to increased activation-induced cell death.[27] There is increasing evidence that the affinity-efficacy relationship follows a bell-shaped form[28] and that efficacy decreases with increasing affinity beyond a threshold.[29,30] Thus, careful selection of the scFv is necessary to allow maximum efficacy and manageable toxicity. A multi-scale system pharmacokinetic (PK)-pharmacodynamic (PD) model[30] built on in vitro data and characterizing the quantitative relationship among CAR-affinity, antigen abundance, tumor cell depletion, and CAR-T cell expansion is available to support the optimization of a CAR's affinity within an MIDD program.

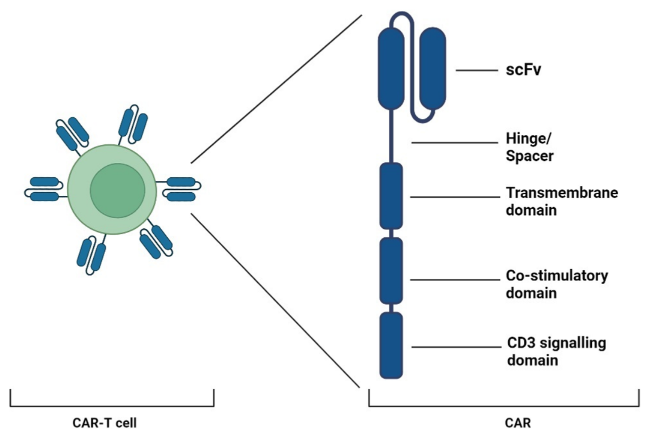


FIGURE 3 CAR-T cell and components of a second-generation CAR. CAR, chimeric antigen receptor; scFv, single chain fragment variable. Created with BioRender.com.

CAR design: Hinge/spacer domain

The hinge/spacer domain of a CAR connects the antigen-binding scFv with the intracellular part of the CAR via the transmembrane domain. Its length determines the distance between the CAR-T and the target cell and can have a profound impact on the CAR-T cell efficacy.[31] Experimental studies have shown that a minimum distance between the CAR and target cell is required for optimal efficacy.[32] This distance can be the natural result of the epitope being located at a sufficient distance from the target cell membrane. If an epitope is located close to the target cell membrane, the required distance can be introduced by the addition of a hinge/spacer into the CAR. The optimal length of a hinge/spacer therefore seems to be dependent on the position of the epitope on the target cell with respect to the cell membrane (e.g., epitopes located close to the cell membrane will require a hinge/spacer while epitopes expressed at a sufficient distance from the target cell will most likely not require one). To the best of our knowledge, no published computational CAR-T cell model currently incorporates the spatial relationship between CAR-T cell and epitope, however, its incorporation in existing physiologically-based PK (PBPK) models, such as the multiscale PK/PD model cited above[30] should be feasible.

CAR design: Co-stimulatory domain

The co-stimulatory domain of a CAR has been reported to influence both expansion and persistence of CAR-T cells. The domains currently used in commercial second-generation CAR-T cell products are CD28 (axicabtagene ciloleucel and brexucabtagene autoleucel) and 41BB/CD137 (tisagenlecleucel, idecabtagene vicleucel, lisocabtagene maraleucel, and ciltacabtagene autoleucel; (Table 1). Previously, the CD28 domain was associated with stronger initial expansion and shorter persistence, whereas the 41BB domain was associated with lower initial expansion but longer persistence.[33] A recent review paper, however, concluded that the efficacy and toxicity associated with either of the two domains are largely the same and that studies designed to compare the efficacy of both domains gave variable results and were often confounded.[34] To obtain a reliable head-to-head comparison of co-stimulatory domains, CAR products with different co-stimulatory domains but otherwise identical constructs and manufacturing processes should be investigated. Combinations of both CD28 and 41BB/CD137 are investigated in third-generation CAR-T cell constructs, however, the combination of several co-stimulatory domains in tandem may, counter-intuitively, lead to inferior persistence and function,[35,36] perhaps due to increased frequencies of activation-induced cell death.[37] Other co-stimulatory domains than CD28 and 41BB/CD137, such as OX40, ICOS, and CD27, are under preclinical investigation and could become clinically relevant in the future.[34]

CAR design: Characterization of element combinations

With multiple options for each of the CAR's components, the effect of their combination is an important next question. The relevance of taking combination effects into account is highlighted by the example of a study investigating the impact of replacing a long scFv linker (connecting the variable heavy and light chains in the scFv) with a short one.[38] In contrast to the long scFv linker, the short version resulted in receptor homodimerization and antigen-independent tonic signaling, which increased the function of CAR-T cells engineered with a 4-1BB co-stimulating domain. Importantly, it had the opposite effect on CAR-T cells engineered with a CD28 co-stimulatory domain, most likely due to the tonic signaling resulting in cell exhaustion.[38] This example underlines that CAR elements should always be assessed as part of the whole receptor construct. Mechanistic models can be used to characterize and extrapolate potential negative, additive, or synergistic combination effects and support rational CAR development.[39] As more CAR designs are being developed, models characterizing the interplay between different receptor components can continually be updated.

DRUG DEVELOPMENT

Manufacturing

The starting material for the autologous CAR-T cell manufacturing process, the apheresis product, comprises the peripheral blood mononuclear cells collected from a patient's blood. After collection, the apheresis product may be cryopreserved before being sent to the manufacturing unit. The steps performed there include the initial treatment of the apheresis product, the incorporation of the CAR into the T cells, the ex vivo expansion of the newly generated CAR-T cells, and the cryopreservation of the final CAR-T cell product for shipment to the treatment center.

The initial treatment of the apheresis product includes the isolation of T cells from buffer and other immune cells. For some products (currently only approved product: brexucabtagene autoleucel; Table 1), a dedicated T cell enrichment step is performed. This step aims to reduce the likelihood of retaining CD19-expressing tumor cells in the medium, as these could result in early exhaustion of the CAR-T cells by providing intense proliferation signals during the ex vivo expansion phase.[40] After isolation and optional enrichment, the T cells are activated, transduced with a vector integrating the CAR, and expanded for usually 9–14 days.[41] The different vectors available for use in the CAR's integration as well as their advantages and disadvantages have recently been reviewed in detail elsewhere.[42]

Some products (currently only approved product: lisocabtagene maraleucel; Table 1) separate $CD4^+$ from $CD8^+$ T cells prior to the ex vivo expansion phase for later administration in a 1:1 ratio, assuming optimal efficacy with equal proportions of $CD4^+$ and $CD8^+$ T cells. Whereas two model-based analyses identified $CD4^+$ CAR-T cells to expand more slowly in vivo than their $CD8^+$ counterparts,[43,44] manufacturing CAR-T cells with a defined CD4-to-CD8 ratio of 1:1 has shown beneficial efficacy results.[45]

There are several factors influencing the CAR-T cell product characteristics, including the CAR-T cell phenotype, during the ex vivo expansion stage: the stimulation of cells and its dose and duration, the cytokines added to the expansion medium and their concentrations, and the optional addition of co-medication to the expansion medium. The last point is reviewed in detail elsewhere[46] and therefore not covered in this paper.

Stimulation of cells during ex vivo expansion and ex vivo expansion duration

To induce ex vivo expansion, the CAR-T cells are stimulated with antigen-presenting antibodies or other artificial antigen-presenting cells (APCs).[46] Usually, the CD3 antigen and a co-stimulating domain, such as CD28, are targeted. Not all stimulation is equally effective, and design elements impacting CAR-T cell expansion, phenotype shift, receptor expression, and cytotoxic potential include the location of the stimulating antibodies in the medium (e.g., soluble vs. bead-bound) or the material, the size, and the shape of the artificial APC.[46] In addition, the dose and duration of the stimulation can impact the phenotypic composition of the infusion product, as longer expansion durations have been reported to result in a higher percentage of a more differentiated phenotype[47,48] and different starting materials (i.e., isolated T cells from patients' apheresis products) might require different doses of stimulation to retain a less-differentiated phenotype. A proof of concept for the model-based selection of a patient-specific stimulation dose to achieve a desired CAR-T cell phenotype has recently been presented.[22] In this work, the authors developed a random forest machine learning classifier which successfully predicted the stimulation dose required to generate a desired CAR-T cell product phenotype using the CAR-T cell phenotype in a patient's apheresis product as input. The ability to calibrate the stimulation dose to a patient's phenotypic composition in the apheresis product is of high relevance because many patients' T cell populations in the apheresis product are primarily of an effector phenotype with poor fitness and at high risk for over-stimulation and exhaustion.[22] Alternatively, new manufacturing methods, for example, based on the optimization of the serum concentration and the surface area-to-volume ratio in the medium, may allow for omission of the in vitro activation and ex vivo expansion steps altogether.[49]

Cytokines in the ex vivo expansion medium

Along with stimulation of the T cell and a co-stimulating receptor, the cytokines added to the cultivation medium are important factors determining the CAR-T cells' phenotype shift during ex vivo expansion. Commonly used cytokines, such as interleukin-2 (IL-2), interleukin-7 (IL-7), interleukin-15 (IL-15), and interleukin-21 (IL-21), belong to the γ-chain receptor family. IL-2 is currently most often applied due to its well-known characteristic of stimulating strong and rapid T cell proliferation. A disadvantage of IL-2, however, is its effect to induce differentiation toward a terminally differentiated, often easily exhausted effector phenotype with low proliferation potential[48,50] as well as populations of regulatory T cells, most prominent during reconstitution after lymphodepletion. IL-7 and IL-15, in contrast, are homeostatic cytokines physiologically regulating the slow proliferation of naïve and memory T cells in absence of target stimulation. As additives in the ex vivo expansion medium, compared to IL-2, they have been reported to result in more efficient expansion and less apoptosis while maintaining a less-differentiated memory cell phenotype and better long-term tumor control.[50] Although IL-2 is usually applied alone, IL-7 and IL-15 may be best used together, as IL-15 primarily induces strong expansion and IL-7 primarily ensures the maintenance of a less differentiated phenotype.[51] Finally, IL-21 has been reported to induce a naïve-to-memory phenotype with high expression of CD28, allowing increased in vivo proliferation and survival compared to IL-2[52] and even IL-15[53] that has led to extended in vivo persistence commensurate with durable clinical responses.[54–56]

In addition to the choice of the cytokine, its concentration can have a significant impact on the CAR-T cell expansion and phenotype shift.[48,52] Kaartinen et al. found that the concentration of IL-2 in the expansion medium was directly related to the increase of the proportion of terminally differentiated effector T cells and the decrease of early differentiated memory T cells in the expansion product.[37] In addition, a bell-shaped relationship was identified for the effect of IL-21 on the expansion of cytotoxic lymphocytes: throughout a dose range of 0.1–100 ng/mL, 30 ng/mL provided optimal stimulation, whereas 100 ng/mL showed an inhibitory effect on the cell expansion.[52] In-depth overviews of the impact of cytokines at different concentrations on the CAR-T cell expansion and phenotype composition are provided in other dedicated review articles.[46,57,58]

Considering the manifold possibilities to influence the CAR-T cell phenotype (and with this the post-infusion expansion capacity) during CAR-T cell manufacturing, an abundance of options to combine design decisions regarding T cell activation, stimulation, expansion duration, and expansion conditions exist. Within an MIDD program, PBPK/quantitative systems pharmacology (QSP),[30,59] population PK/PD,[1,43,44,60] and emerging machine learning[22] approaches can be applied to incorporate the quantitative findings obtained in carefully chosen in vitro experiments investigating the impact of such design choices. The generated models can then be leveraged to perform hypothesis-generating simulations regarding the interactions of the actionable variables in the manufacturing phase, followed by model-informed design of the hypothesis-testing experiments.

Preclinical development

Prediction of in vivo efficacy based on in vitro data

Several in vitro and preclinical in vivo models exist for the prediction of clinical efficacy and toxicity of CAR-T cell therapy.[61,62] In vitro cell killing assays are most frequently used to assess antitumor activity, however, their translation into in vivo expansion, persistence, and efficacy potential is far from optimal.[33,38,62] In a study assessing the in vitro, preclinical, and clinical in vivo antitumor effects for CAR-T cells targeting the GD2 antigen (commonly expressed on solid tumors), the cells showed strong antitumor efficacy in vitro but not in vivo.[33] When assessing the cells' fate during ex vivo expansion, signs of exhaustion and low cytokine production due to tonic receptor signaling were observed. The authors concluded from this that commonly used cytotoxicity assays were poor predictors for the in vivo efficacy, as CAR-T cells prone to exhaustion retain their cytolytic capacity in vitro despite their poor efficacy in vivo. To better test for the risk of exhaustion, the in vitro tests could be performed for a prolonged period, during which fresh tumor cells would repeatedly be added to the medium to maintain a defined effector-to-target ratio.[63] A proposed alternative in vitro predictor for clinical antitumor efficacy is the ability of CAR-T cells to produce polyfunctional cytokines early after antigen exposure.[33,38]

In addition, the model-informed analysis of CAR-T cell proliferation and exhaustion using real-time in vitro killing assay data could help to characterize the relationship between target cell-related features (e.g., tumor growth rate and level of antigen expression) and the properties (e.g., proliferation rate, exhaustion rate, and killing rate) of a new CAR-T cell construct.[64] Mathematical models may moreover help to predict the risk for tonic signaling based on the CAR design during the drug discovery stage, thereby reducing the risk of aborting development at the transition from the in vitro to the preclinical in vivo stage.[62]

Finally, as successfully demonstrated in a bench-to-bedside translation for idecabtagene ciloleucel, in vitro killing assay data can be used to determine the CAR-T cell potency on the cell-to-cell level, supporting the subsequent development of (semi-)mechanistic preclinical and clinical tumor growth inhibition models.[65] Concretely, the authors facilitated their bench-to-bedside translation through the sequential integration of different sources of data generated during early development. First, in vitro cell killing data was used to develop a cell-level PD model characterizing the in vitro activity of the CAR-T cells against tumor cell lines. Next, the cell-level in vitro potency estimate was used as a fixed parameter, describing the number of CAR-Target complexes per tumor cell required to achieve 50% of the maximum killing rate, as part of PK/PD models fitted to in vivo preclinical and clinical datasets. This approach facilitated successful preclinical and clinical predictions for idecabtagene ciloleucel and could be applied for other CAR-T cell candidates in the future.

Prediction of the clinical efficacy based on preclinical in vivo data

After demonstrating antitumor activity in vitro, promising CAR-T cell candidates are next tested in preclinical animal models. Both immune-compromised and immune-competent mice are used, each with their own advantages and disadvantages. Immune-compromised mice, such as NSG mouse models, lack cells crucial for the function of both the innate and the adaptive immune system, and therefore allow the implantation of patient-derived xenografts.[62] Studying the interplay of CAR-T cell candidates with original human tumors is imperative to understanding their behavior in a living organism. To learn about the exposure-response profile, robustness against exhaustion, as well as the interindividual variability inherent to individual starting materials and tumors, different donor cells (starting material), dose levels, xenografts (tumors), and effector-to-target ratios should be tested.[65] A disadvantage of immune-compromised mice is the inability to study the interplay between CAR-T cells and the host immune system. This is important as cells of the endogenous immune system have been reported to be relevant for both the efficacy[66] and toxicity[67] of CAR-T cells. Moreover, potential on-target off-tumor toxicities cannot be detected if the target antigen is not conserved between humans and mice.

Immune-competent mouse models allow one to study the interaction between CAR-T cells and the host immune system, including the tumor micro-environment. Moreover, immunomodulatory effects of lymphodepletion[68] and combination regimens with other immune-targeting agents[69] can be investigated. Available immune-competent mouse models include syngeneic mice with implanted histocompatible tumors, genetically engineered mice, and humanized mice with a partially reconstituted human immune system.[61,62] A detailed description and comparison of the advantages and disadvantages of the different types of immune-competent mouse models is provided elsewhere,[61,62] however, it should be noted here, that there is currently no optimal preclinical in vivo model and, at best, a qualified approximation will be made that will in part be dependent on the specific research question, availability of materials (e.g., human tumor tissue), funding resources, and understanding of potential shortcomings of a given model.

In addition to providing information on the in vivo expansion, persistence, efficacy, and safety, both immune-compromised and immune-competent mice may allow one to study the distribution and accumulation of the CAR-T cells in different organs. This information may be used to advance previously developed in vitro-based cell-level models into semi-mechanistic[65] or PBPK/PD models.[30,59]

Clinical development

Translation of early biomarker response to clinical outcomes

Several biomarkers for the prediction of clinical outcomes have been proposed based on phase I and phase II clinical or real-world data. High maximum concentrations in peripheral blood (C_{max})[9,10,21,70–73] and AUC in the first month after infusion ($AUC_{0–28d}$)[10,21,70,71] have been associated with better response and/or response duration for several CAR-T cell products. High correlations between C_{max} and $AUC_{0–28d}$ are usually observed,[10,72] which implies that either could act as a relevant exposure metric.

Although CAR-T cell expansion is often positively correlated with tumor size,[21,43] a high baseline tumor burden has also been identified as a negative predictor for clinical outcome.[7,21] Based on the hypothesis that a sufficient effector-to-target ratio needs to be reached for a successful antitumor response, the ratio of CAR-T cell C_{max}/baseline tumor burden could therefore be an alternative response predictor to C_{max} alone.[21,43,74]

Mixed results have been reported for the association between CAR-T cell persistence, measured as the AUC integrated from the time of infusion until the last observation (AUC_{last}), and outcome. Whereas positive correlations between AUC_{last} and response duration have been identified,[70,73] durable responses despite undetectable CAR-T cell transgene levels in blood are observed as well. Of note, relationships between CAR-T cell persistence and response duration have mostly been observed for CAR products using the 41BB co-stimulatory domain, whereas no such relationships could be identified for products using the CD28 domain, possibly attributable to 4-1BB-specific downstream signaling.

As mentioned in the introduction to this paper, the phenotype composition in the infusion product seems to be an essential determinant of the CAR-T cell expansion and antitumor efficacy, with low-differentiation phenotypes such T_N, T_{SCM}, and T_{CM} being associated with favorable response.[18–21] Notably, supporting the importance of the phenotypic composition, two independent analyses, one of them model-based, identified the above-suggested biomarker of CAR-T cell C_{max}/baseline tumor burden ratio to be of increased predictive performance when using only the naïve instead of all CAR-T cells in the denominator.[21,43]

Recently, an analysis of clinical data from approved CAR-T cell products applying a machine learning workflow (including particle swarm optimization, principal component analysis, and single-sample gene set enrichment analysis) identified cell-intrinsic differences, such as a proliferative capacity and death rates as well as the cytotoxicity potential to be of superior predictive performance than phenotype.[74] Using bulk gene expression data of pre-infusion CAR-T cell products, the authors identified an enrichment of memory cell signatures, heightened proliferative and inflammatory signaling, and lack of exhaustion markers to be positively correlated with CAR-T cell expansion, persistence, and antitumor response.

As highlighted above, there are several questions and MIDD applications that are specific to CAR-T cell therapy. The traditional exposure-response focused MIDD applications may be less relevant for CAR-T cell therapy, however, in other aspects, the CAR-T cell therapy development programs can benefit from the same MIDD applications as other therapeutic modalities in the same indication. This includes applications such as linking early response biomarkers to clinical end points[75–77] and using such predictions to evaluate probability of success against a predefined target and/or in contrast to an active comparator.[78–80]

CLINICAL PRACTICE

Compared to the number of actionable variables in the drug discovery and drug development phases, there are relatively few in the clinical practice stage. Clinical actionable variables include an optional bridging therapy between the previous line and CAR-T cell therapy as well as extrinsic factors, such as immunomodulation before or after T cell infusion (including but not limited to lymphodepleting chemotherapy regimen, post-infusion cytokines, etc.). In the future, model-based selection of patient subgroups who might benefit the most from CAR-T cell therapy could be possible.

A high baseline tumor burden has been associated with poor CAR-T cell efficacy[7,43] and patients with aggressive tumors often need disease control during the ~30 days between apheresis and CAR-T cell infusion. By reducing the tumor size prior to CAR-T cell infusion, a bridging chemotherapy between apheresis and CAR-T cell therapy can improve the in vivo CAR-T cell survival conditions and reduce the severity of CAR-T cell associated toxicities.[81] Care should be taken to adjust the treatment intensity to the patient's overall condition as a too high intensity can result in immunosuppression and poor prognosis. Moreover, mixed results on the effect of bridging therapy on the efficacy and toxicity of CAR-T cell treatment have been reported, and the interpretation of these findings is challenging, as bridging therapy is usually administered to patients who have a poor prognosis to begin with.[81] Further studies should be performed to investigate the impact of different bridging therapy regimens on CAR-T cell therapy outcome in different patient subpopulations.

The preconditioning lymphodepleting chemotherapy administered in the days before CAR-T cell infusion has several positive effects: it modulates the tumor microenvironment and removes cytokine sinks while simultaneously stimulating the release of homeostatic cytokines, such as IL-7 and IL-15.[82,83] Patients usually receive a combination of high-dose cyclophosphamide and fludarabine over 3–4 days, however, other regimens such as bendamustine alone exist. Recently, a modeling and simulation framework to test the impact of different preconditioning regimens on CAR-T cell treatment success has been proposed.[84] The authors identified the optimal regimen to be dependent on a patient's tumor burden and intended CAR-T cell dose. Moreover, they identified the rest period between lymphodepleting chemotherapy and CAR-T cell infusion to be tumor growth rate-dependent and important for the preconditioning's success. The proposed model could be the basis for a personalized lymphodepleting regimen in the future.

Focusing on the currently most common lymphodepleting regimen of cyclophosphamide-fludarabine, exposure-response relationships have been observed for both drugs. In a study applying cyclophosphamide alone, a high dose of cyclophosphamide was associated with significantly better expansion and survival compared to a low dose.[85] Similarly, two independent studies applying cyclophosphamide and fludarabine in combination identified fludarabine exposure to be directly associated with clinical outcome.[86,87] Notably, in both fludarabine studies, all patients had received the same body size area-normalized dose of 30 mg/m^2 yet a wide interindividual variability in observed plasma fludarabine concentrations was observed and AUC efficacy thresholds of 13.8[86] and 14.0 mg h/L[87] were identified.

CAR-T cell therapy as part of a combination treatment strategy is currently being investigated in preclinical and early clinical trials. A detailed review of all explored combinations is beyond the scope of this paper but has been the focus of a different paper.[88] No combination strategy is currently approved by a regulatory agency and further studies will have to be performed before the first combined treatment protocol will become available.

Compared to the drug discovery and drug development phases, which will be best supported by mechanistic PBPK/QSP approaches, a population-based approach to quantify the impact of the described actionable variables becomes more relevant in the clinical practice stage. Data-driven population (nonlinear mixed-effects) models[1,44,60,89] can be used to describe the general trend in often sparse data, the variability within the population, and potential covariates influencing different kinetic/dynamic processes. Moreover, they can be used to compare parameters across drug products, diseases, patient subpopulations, and response status.[44] Other population-based modeling and simulation approaches[12,90] applied to clinical data can additionally help to understand the complex CAR-T cell – tumor interactions and propose underlying factors for varying treatment responses. Models at the interface of QSP and population PK/PD modeling[43,65] add a more mechanistic layer while retaining the advantages of a population approach and can be used to generate hypotheses regarding underlying mechanisms of identified significant covariates. In addition, machine learning algorithms have added to the repertoire of available modeling approaches to identify predictors for clinical response from clinical data.[74]

FUTURE PERSPECTIVES

All currently approved CAR-T cell products are autologous (i.e., manufactured from a patient's own T cells), equipped with second-generation CARs, and targeting hematological malignancies. The development of CAR-T cells for solid tumors has so far been largely unsuccessful, due to several reasons, including limited penetration into tumor tissue as well as low antitumor efficacy and persistence. However, a variety of approaches to overcome these challenges are being explored, such as utilizing different autologous or allogeneic cell types, using different natural or artificial receptors, or modulating the tumor microenvironment.[91] These novel approaches might show deviations from the relatively well-characterized kinetics and dynamics of the currently approved autologous second-generation CAR-T cell products. A MIDD program can help to quantitatively evaluate the impact of these changes on CAR-T cell expansion, persistence, phenotype, efficacy, and safety, and allow for more efficient candidate comparison to accelerate the development process.

ACKNOWLEDGMENTS
Figures 1 and 3 were created with BioRender.com.

FUNDING INFORMATION
No funding was received for this work.

CONFLICT OF INTEREST STATEMENT
C.Y. is on scientific advisory boards in the field of cellular therapy but none that is directly involved in the use of computational models. A.M.M., P.A.M., and M.B. are employees of Pharmetheus AB and are paid consultants to multiple pharmaceutical companies. M.B. and P.A.M. own stock in Pharmetheus AB.

ORCID
Anna M. Mc Laughlin https://orcid.org/0000-0002-5936-1877
Cassian Yee https://orcid.org/0000-0002-4429-7307
Martin Bergstrand https://orcid.org/0000-0002-0295-1227

REFERENCES
1. Stein AM, Grupp SA, Levine JE, et al. Tisagenlecleucel model-based cellular kinetic analysis of chimeric antigen receptor–T cells. *CPT: Pharmacometrics Syst Pharmacol*. 2019;8:285-295.
2. Huang W, Li J, Liao MZ, et al. Clinical pharmacology perspectives for adoptive cell therapies in oncology. *Clin Pharmacol Ther*. 2021;112:968-981. doi:10.1002/cpt.2509
3. Gattinoni L, Klebanoff CA, Restifo NP. Paths to stemness: building the ultimate antitumour T cell. *Nat Rev Cancer*. 2012;12:671-684.
4. Xiong Y, Bosselut R. CD4–CD8 differentiation in the thymus: connecting circuits and building memories. *Curr Opin Immunol*. 2012;24:139-145.
5. van de Donk NWCJ, Agha ME, Cohen AD, et al. Biological correlative analyses and updated clinical data of ciltacabtagene autoleucel (cilta-cel), a BCMA-directed CAR-T cell therapy, in patients with multiple myeloma (MM) and early relapse after initial therapy: CARTITUDE-2, cohort B. *J Clin Oncol*. 2022;40:8029.
6. Locke FL, Ghobadi A, Jacobson CA, et al. Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): a single-arm, multicentre, phase 1–2 trial. *Lancet Oncol*. 2019;20:31-42.
7. Dean EA, Mhaskar RS, Lu H, et al. High metabolic tumor volume is associated with decreased efficacy of axicabtagene ciloleucel in large B-cell lymphoma. *Blood Adv*. 2020;4:3268-3276.
8. Rodriguez-Garcia A, Palazon A, Noguera-Ortega E, Powell DJ, Guedan S. CAR-T cells hit the tumor microenvironment: strategies to overcome tumor escape. *Front Immunol*. 2020;11:1109.
9. Frey NV, Gill S, Hexner EO, et al. Long-term outcomes from a randomized dose optimization study of chimeric antigen receptor modified T cells in relapsed chronic lymphocytic leukemia. *J Clin Oncol*. 2020;38:2862-2871.
10. Connarn JN, Witjes H, van Zutphen-van Geffen M, et al. Characterizing the exposure–response relationship of idecabtagene vicleucel in patients with relapsed/refractory multiple myeloma. *CPT Pharmacometrics Syst Pharmacol*. 2023;1-11.
11. Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N Engl J Med*. 2018;378:439-448.
12. Kimmel GJ, Locke FL, Altrock PM. The roles of T cell competition and stochastic extinction events in chimeric antigen receptor T cell therapy. *Proc Biol Sci*. 2021;288:20210229.
13. Lalonde RL, Kowalski KG, Hutmacher MM, et al. Model-based drug development. *Clin Pharmacol Ther*. 2007;82:21-32.
14. Milligan PA, Brown MJ, Marchant B, et al. Model-based drug development: a rational approach to efficiently accelerate drug development. *Clin Pharmacol Ther*. 2013;93:502-514.
15. Center for Drug Evaluation and Research. CDER conversation: model informed drug development. FDA. 2019. https://www.fda.gov/drugs/news-events-human-drugs/cder-conversation-model-informed-drug-development
16. D'Argenio DZ. Optimal sampling times for pharmacokinetic experiments. *J Pharmacokinet Biopharm*. 1981;9:739-756.
17. Hooker A, Vicini P. Simultaneous population optimal design for pharmacokinetic-pharmacodynamic experiments. *AAPS J*. 2005;7:E759-E785.
18. Monfrini C, Stella F, Aragona V, et al. Phenotypic composition of commercial anti-CD19 CAR T cells affects *in vivo* expansion and disease response in patients with large B-cell lymphoma. *Clin Cancer Res*. 2022;28:3378-3386.
19. Deng Q, Han G, Puebla-Osorio N, et al. Characteristics of anti-CD19 CAR T cell infusion products associated with efficacy and toxicity in patients with large B cell lymphomas. *Nat Med*. 2020;26:1878-1887.

20. Fraietta JA, Lacey SF, Orlando EJ, et al. Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. *Nat Med*. 2018;24:563-571.
21. Locke FL, Rossi JM, Neelapu SS, et al. Tumor burden, inflammation, and product attributes determine outcomes of axicabtagene ciloleucel in large B-cell lymphoma. *Blood Adv*. 2020;4:4898-4911.
22. Zhang DKY, Adu-Berchie K, Iyer S, et al. Enhancing CAR-T cell functionality in a patient-specific manner. *Nat Commun*. 2023;14:506.
23. June CH, Maus MV, Plesa G, et al. Engineered T cells for cancer therapy. *Cancer Immunol Immunother*. 2014;63:969-975.
24. Strati P, Neelapu SS. Chimeric antigen receptor–engineered T cell therapy in lymphoma. *Curr Oncol Rep*. 2019;21:38.
25. Greenman R, Pizem Y, Haus-Cohen M, et al. Phenotypic models of CAR T-cell activation elucidate the pivotal regulatory role of CAR downmodulation. *Mol Cancer Ther*. 2021;20:946-957.
26. Ghorashian S, Kramer AM, Onuoha S, et al. Enhanced CAR T cell expansion and prolonged persistence in pediatric patients with ALL treated with a low-affinity CD19 CAR. *Nat Med*. 2019;25:1408-1414.
27. Watanabe K, Terakura S, Uchiyama S, et al. Excessively high-affinity single-chain fragment variable region in a chimeric antigen receptor can counteract T-cell proliferation. *Blood*. 2014;124:4799.
28. Greenman R, Pizem Y, Haus-Cohen M, et al. Shaping functional avidity of CAR T cells: affinity, avidity, and antigen density that regulate response. *Mol Cancer Ther*. 2021;20:872-884.
29. Oren R, Hod-Marco M, Haus-Cohen M, et al. Functional comparison of engineered T cells carrying a native TCR versus TCR-like antibody–based chimeric antigen receptors indicates affinity/avidity thresholds. *J Immunol*. 2014;193:5733-5743.
30. Singh AP, Zheng X, Lin-Schmidt X, et al. Development of a quantitative relationship between CAR-affinity, antigen abundance, tumor cell depletion and CAR-T cell expansion using a multiscale systems PK-PD model. *mAbs*. 2020;12:e1688616.
31. Irving M, Vuillefroy De Silly R, Scholten K, Dilek N, Coukos G. Engineering chimeric antigen receptor T-cells for racing in solid tumors: don't forget the fuel. *Front Immunol*. 2017;8:267.
32. Guest RD, Hawkins RE, Kirillova N, et al. The role of extracellular spacer regions in the optimal Design of Chimeric Immune Receptors: evaluation of four different scFvs and antigens. *J Immunother*. 2005;28:203-211.
33. Long AH, Haso WM, Shern JF, et al. 4-1BB costimulation ameliorates T cell exhaustion induced by tonic signaling of chimeric antigen receptors. *Nat Med*. 2015;21:581-590.
34. Cappell KM, Kochenderfer JN. A comparison of chimeric antigen receptors containing CD28 versus 4-1BB costimulatory domains. *Nat Rev Clin Oncol*. 2021;18:715-727.
35. Abate-Daga D, Lagisetty KH, Tran E, et al. A novel chimeric antigen receptor against prostate stem cell antigen mediates tumor destruction in a humanized mouse model of pancreatic cancer. *Hum Gene Ther*. 2014;25:1003-1012.
36. Milone MC, Fish JD, Carpenito C, et al. Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy in vivo. *Mol Ther*. 2009;17:1453-1464.
37. Hombach AA, Rappl G, Abken H. Arming cytokine-induced killer cells with chimeric antigen receptors: CD28 outperforms combined CD28–OX40 "super-stimulation". *Mol Ther*. 2013;21:2268-2277.
38. Singh N, Frey NV, Engels B, et al. Antigen-independent activation enhances the efficacy of 4-1BB-costimulated CD22 CAR T cells. *Nat Med*. 2021;27:842-850.
39. Wicha SG, Chen C, Clewe O, Simonsson USH. A general pharmacodynamic interaction model identifies perpetrators and victims in drug interactions. *Nat Commun*. 2017;8:2129.
40. Mian A, Hill BT. Brexucabtagene autoleucel for the treatment of relapsed/refractory mantle cell lymphoma. *Expert Opin Biol Ther*. 2021;21:435-441.
41. Zhang H, Zhao P, Huang H. Engineering better chimeric antigen receptor T cells. *Exp Hematol Oncol*. 2020;9:34.
42. Irving M, Lanitis E, Migliorini D, Ivics Z, Guedan S. Choosing the right tool for genetic engineering: clinical lessons from chimeric antigen receptor-T cells. *Hum Gene Ther*. 2021;32:1044-1058.
43. Mueller-schoell A, Puebla-Osorio N, Michelet R, et al. Early survival prediction framework in cd19-specific car-t cell immunotherapy using a quantitative systems pharmacology model. *Cancer*. 2021;13:2782.
44. Liu C, Ayyar VS, Zheng X, et al. Model-based cellular kinetic analysis of chimeric antigen receptor-T cells in humans. *Clin Pharmacol Ther*. 2021;109:716-727.
45. Turtle CJ, Hanafi LA, Berger C, et al. CD19 CAR–T cells of defined CD4+:CD8+ composition in adult B cell ALL patients. *J Clin Investig*. 2016;126:2123-2138.
46. Liu Y, Sperling AS, Smith EL, Mooney DJ. Optimizing the manufacturing and antitumour response of CAR T therapy. *Nat Rev Bioeng*. 2023;1:271-285. doi:10.1038/s44222-023-00031-x
47. Alvarez-Fernández C, Escribà-Garcia L, Vidal S, Sierra J, Briones J. A short CD3/CD28 costimulation combined with IL-21 enhance the generation of human memory stem T cells for adoptive immunotherapy. *J Transl Med*. 2016;14:214.
48. Kaartinen T, Luostarinen A, Maliniemi P, et al. Low interleukin-2 concentration favors generation of early memory T cells over effector phenotypes during chimeric antigen receptor T-cell expansion. *Cytotherapy*. 2017;19:689-702.
49. Ghassemi S, Durgin JS, Nunez-Cruz S, et al. Rapid manufacturing of non-activated potent CAR T cells. *Nat Biomed Eng*. 2022;6:118-128.
50. Zhou J, Jin L, Wang F, Zhang Y, Liu B, Zhao T. Chimeric antigen receptor T (CAR-T) cells expanded with IL-7/IL-15 mediate superior antitumor effects. *Protein Cell*. 2019;10:764-769.
51. Cieri N, Camisa B, Cocchiarella F, et al. IL-7 and IL-15 instruct the generation of human memory stem T cells from naive precursors. *Blood*. 2013;121:573-584.
52. Li Y, Bleakley M, Yee C. IL-21 influences the frequency, phenotype, and affinity of the antigen-specific CD8 T cell response. *J Immunol*. 2005;175:2261-2269.
53. Hinrichs CS, Spolski R, Paulos CM, et al. IL-2 and IL-21 confer opposing differentiation programs to CD8+ T cells for adoptive immunotherapy. *Blood*. 2008;111:5326-5333.
54. Chapuis AG, Desmarais C, Emerson R, et al. Tracking the fate and origin of clinically relevant adoptively transferred CD8+ T cells in vivo. *Sci Immunol*. 2017;2:eaal2568.
55. Chapuis AG, Lee SM, Thompson JA, et al. Combined IL-21–primed polyclonal CTL plus CTLA4 blockade controls

refractory metastatic melanoma in a patient. *J Exp Medicine*. 2016;213:1133-1139.
56. Chapuis AG, Roberts IM, Thompson JA, et al. T-cell therapy using Interleukin-21–primed cytotoxic T-cell lymphocytes combined with cytotoxic T-cell lymphocyte Antigen-4 blockade results in Long-term cell persistence and durable tumor regression. *J Clin Oncol*. 2016;34:3787-3795. doi:10.1200/JCO.2015.65.5142
57. Watanabe N, Mo F, McKenna MK. Impact of manufacturing procedures on CAR T cell functionality. *Front Immunol*. 2022;13:876339.
58. Silveira CRF, Corveloni AC, Caruso SR, et al. Cytokines as an important player in the context of CAR-T cell therapy for cancer: their role in tumor immunomodulation, manufacture, and clinical implications. *Front Immunol*. 2022;13:947648.
59. Tsai C-H, Singh AP, Xia CQ, Wang H. Development of minimal physiologically-based pharmacokinetic-pharmacodynamic models for characterizing cellular kinetics of CAR T cells following local deliveries in mice. *J Pharmacokinet Pharmacodyn*. 2022;49:525-538. doi:10.1007/s10928-022-09818-8
60. Ogasawara K, Dodds M, Mack T, Lymp J, Dell'Aringa J, Smith J. Population cellular kinetics of lisocabtagene maraleucel, an autologous CD19-directed chimeric antigen receptor T-cell product, in patients with relapsed/refractory large B-cell lymphoma. *Clin Pharmacokinet*. 2021;60:1621-1633.
61. Si X, Xiao L, Brown CE, Wang D. Preclinical evaluation of CAR T cell function: in vitro and In vivo models. *Int J Mol Sci*. 2022;23:3154.
62. Guedan S, Luu M, Ammar D, et al. Time 2EVOLVE: predicting efficacy of engineered T-cells – how far is the bench from the bedside? *J Immunother Cancer*. 2022;10:e003487.
63. Kiesgen S, Messinger JC, Chintala NK, Tano Z, Adusumilli PS. Comparative analysis of assays to measure CAR T-cell-mediated cytotoxicity. *Nat Protoc*. 2021;16:1331-1342.
64. Sahoo P, Yang X, Abler D, et al. Mathematical deconvolution of CAR T-cell proliferation and exhaustion from real-time killing assay data. *J Roy Soc Interface*. 2020;17:20190734.
65. Singh AP, Chen W, Zheng X, et al. Bench-to-bedside translation of chimeric antigen receptor (CAR) T cells using a multiscale systems pharmacokinetic-pharmacodynamic model: a case study with anti-BCMA CAR-T. *CPT Pharmacometrics Syst Pharmacol*. 2021;10:362-376.
66. Li X, Guo X, Zhu Y, et al. Single-cell transcriptomic analysis reveals BCMA CAR-T cell dynamics in a patient with refractory primary plasma cell leukemia. *Mol Ther*. 2021;29:645-657.
67. Xiao X, Huang S, Chen S, et al. Mechanisms of cytokine release syndrome and neurotoxicity of CAR T-cell therapy and associated prevention and management strategies. *J Exp Clin Cancer Res*. 2021;40:367.
68. Murad JP, Tilakawardane D, Park AK, et al. Pre-conditioning modifies the TME to enhance solid tumor CAR T cell efficacy and endogenous protective immunity. *Mol Ther*. 2021;29:2335-2349.
69. Kato D, Yaguchi T, Iwata T, et al. GPC1 specific CAR-T cells eradicate established solid tumor without adverse effects and synergize with anti-PD-1 ab. *eLife*. 2020;9:e49392.
70. Mueller KT, Maude SL, Porter DL, et al. Cellular kinetics of CTL019 in relapsed/refractory B-cell acute lymphoblastic leukemia and chronic lymphocytic leukemia. *Blood*. 2017;130:2317-2325.
71. Hay KA, Gauthier J, Hirayama AV, et al. Factors associated with durable EFS in adult B-cell ALL patients achieving MRD-negative CR after CD19 CAR T-cell therapy. *Blood*. 2019;133:1652-1663.
72. Awasthi R, Pacaud L, Waldron E, et al. Tisagenlecleucel cellular kinetics, dose, and immunogenicity in relation to clinical factors in relapsed/refractory DLBCL. *Blood Adv*. 2020;4:560-572.
73. Dupouy S, Marchiq I, Derippe T, et al. Clinical pharmacology and determinants of response to UCART19, an allogeneic anti-CD19 CAR-T cell product, in adult B-cell acute lymphoblastic leukemia. *Cancer Res Commun*. 2022;2:1520-1531.
74. Kirouac DC, Zmurchok C, Deyati A, Sicherman J, Bond C, Zandstra PW. Deconvolution of clinical variance in CAR-T cell pharmacology and response. *Nat Biotechnol*. 2023. doi:10.1038/s41587-023-01687-x
75. Netterberg I, Li CC, Molinero L, et al. A PK/PD analysis of circulating biomarkers and their relationship to tumor response in atezolizumab-treated non-small cell lung cancer patients. *Clin Pharmacol Ther*. 2019;105:486-495.
76. Bruno R, Bottino D, de Alwis DP, et al. Progress and opportunities to advance clinical cancer therapeutics using tumor dynamic models. *Clin Cancer Res*. 2020;26:1787-1795.
77. Yu J, Wang N, Kågedal M. A new method to model and predict progression free survival based on tumor growth dynamics. *CPT Pharmacometrics Syst Pharmacol*. 2020;9:177-184.
78. Hampson LV, Bornkamp B, Holzhauer B, et al. Improving the assessment of the probability of success in late stage drug development. *Pharm Stat*. 2022;21:439-459.
79. Lalonde RL, Peck CC. Probability of success: a crucial concept to inform decision making in pharmaceutical research and development. *Clin Pharmacol Ther*. 2022;111:1001-1003.
80. Zierhut M, Bycott P, Gibbs M, Smith B, Vicini P. Ignorance is not bliss: statistical power is not probability of trial success. *Clin Pharmacol Ther*. 2016;99:356-359.
81. Bhaskar ST, Dholaria BR, Sengsayadeth SM, Savani BN, Oluwole OO. Role of bridging therapy during chimeric antigen receptor T cell therapy. *eJHaem*. 2022;3:39-45.
82. Condomines M, Veyrune JL, Larroque M, et al. Increased plasma-immune cytokines throughout the high-dose melphalan-induced Lymphodepletion in patients with multiple myeloma: a window for adoptive immunotherapy. *J Immunol*. 2010;184:1079-1084.
83. Derippe T, Fouliard S, Marchiq I, et al. Mechanistic modeling of the interplay between host immune system, interleukin 7 and UCART19 allogeneic CAR-T cells in adult B-cell acute lymphoblastic leukemia. *Cancer Res Commun*. 2022;2:1532-1544. doi:10.1158/2767-9764.crc-22-0176
84. Owens K, Bozic I. Modeling CAR T-cell therapy with patient preconditioning. *Bull Math Biol*. 2021;83:42.
85. Curran KJ, Margossian SP, Kernan NA, et al. Toxicity and response after CD19-specific CAR T-cell therapy in pediatric/young adult relapsed/refractory B-ALL. *Blood*. 2019;134:2361-2368.
86. Fabrizio VA, Boelens JJ, Mauguen A, et al. Optimal fludarabine lymphodepletion is associated with improved outcomes after CAR T-cell therapy. *Blood Adv*. 2022;6:1961-1968.
87. Dekker L, Calkoen FG, Jiang Y, et al. Fludarabine exposure predicts outcome after CD19 CAR T-cell therapy in children and young adults with acute leukemia. *Blood Adv*. 2022;6:1969-1976.
88. Xiao X, Wang Y, Zou Z, et al. Combination strategies to optimize the efficacy of chimeric antigen receptor T cell

therapy in haematological malignancies. *Front Immunol*. 2022;13:954235.
89. Wu LS, Su Y, Li C, et al. Population-based cellular kinetic characterization of ciltacabtagene autoleucel in subjects with relapsed or refractory multiple myeloma. *Clin Transl Sci*. 2022;15:3000-3011.
90. Brummer AB, Xella A, Woodall R, et al. Data driven model discovery and interpretation for CAR T-cell killing using sparse identification and latent variables. *Front Immunol*. 2023;14:1115536.
91. Rafiq S, Hackett CS, Brentjens RJ. Engineering strategies to overcome the current roadblocks in CAR T cell therapy. *Nat Rev Clin Oncol*. 2020;17:147-167.

How to cite this article: Mc Laughlin AM, Milligan PA, Yee C, Bergstrand M. Model-informed drug development of autologous CAR-T cell therapy: Strategies to optimize CAR-T cell exposure leveraging cell kinetic/dynamic modeling. *CPT Pharmacometrics Syst Pharmacol*. 2023;12:1577-1590. doi:10.1002/psp4.13011